Journal of Medical Microbiology and Infectious Diseases

JoMMID
ISSN: 2345-5349
eISSN: 2345-5330

Sporadic *Stenotrophomonas maltophilia* Respiratory Infections in Cancer Patients

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ARTICLE INFO

Original Article

Keywords: Cancer patients, Immunocompromised, Non-fermenting Gram-negative bacilli, Respiratory infections, Sporadic, *Stenotrophomonas maltophilia*

Received: 18 Apr. 2024
Received in revised form: 28 Apr. 2025
Accepted: 10 May. 2025
DOI: 10.61186/JoMMID.13.1.50

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ABSTRACT

Introduction: *Stenotrophomonas maltophilia* is a bacterium that increasingly causes respiratory and bloodstream infections, particularly in individuals with compromised immune systems, such as cancer patients. This study investigates a series of *S. maltophilia* respiratory infections among cancer patients at a large hospital, aiming to describe the clinical and microbiological characteristics of these cases. **Methods**: We investigated cancer patients presenting with respiratory symptoms between February 1st and February 28th, 2023 in ten of whom *S. maltophilia* isolates were recovered from clinical specimens through microbiological culture. Identification was initially based on colony morphology, oxidase reaction, glucose fermentation, motility, and other biochemical tests, followed by confirmation using the Vitek® 2 system (BioMérieux). Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. **Results**: Ten adult cancer patients (6 females, 4 males) developed *S. maltophilia* pneumonia between February 1st and February 28th, 2023. Eight patients had hematological malignancies, and two had solid tumors. All patients' infections were susceptible to treatment with trimethoprim-sulfamethoxazole, and all patients recovered fully following treatment. **Conclusion:** We identified a cluster of *S. maltophilia* respiratory infections, but it did not meet the criteria for a true outbreak due to the lack of a common source and evidence of transmission between patients. Despite reinforced infection control measures, limitations in surveillance hindered identifying the source and implementing prevention strategies. This cluster emphasizes the need for vigilance in recognizing *S. maltophilia* infections in cancer patients, enabling timely, susceptibility-guided antimicrobial therapy.

INTRODUCTION

S. maltophilia is a ubiquitous, Gram-negative bacillus that is increasingly recognized as a cause of infections in people with compromised immune systems [1, 2]. Initially identified in 1943 and classified as *Pseudomonas maltophilia*, it was later reclassified and renamed *Stenotrophomonas maltophilia* in 1993. This organism has gained clinical significance as an emerging cause of infections among non-fermenting Gram-negative bacteria, alongside *Pseudomonas* and *Acinetobacter* species [2-4].

S. maltophilia is a bacterium that can cause infections in hospitalized patients, particularly those with compromised immune systems [1-6]. Infections usually occur in people with weakened immune systems, such as cancer patients, but can also occur in people with normal immune systems, albeit less often [3, 5]. *S. maltophilia* infections primarily affect the respiratory tract, bloodstream, skin, and urinary tract [1, 4].

S. maltophilia exhibits a predilection for moist environments, facilitating its colonization of water sources within hospital and healthcare settings [1, 2]. It has been found in various water sources and medical equipment, including water distribution systems, medical devices, ventilator circuits, irrigation solutions, intravenous fluids, disinfectant solutions, hand hygiene products, contact lens solutions, hemodialysis water, and nebulizers [4, 5]. As a result, people can get infected with *S. maltophilia* by coming into contact with these contaminated sources [6].

In large hospitals that specialize in cancer care, *S. maltophilia* poses a significant nosocomial threat to patients due to its potential for opportunistic infections. Quick and effective treatment of *S. maltophilia* infections,

[DOI: 10.61186/JoMMID.13.1.50]

primarily with trimethoprim-sulfamethoxazole, is essential for patients with weakened immune systems. This study aims to analyze the clinical, microbiological, and epidemiological characteristics of a cluster of sporadic *S. maltophilia* respiratory infections in cancer patients over a short span of one month at our institution.

MATERIAL AND METHODS

Study design and setting. This study presents a case series analysis conducted within the Department of Microbiology at a large tertiary cancer center in southern India. We included 410 respiratory specimens collected from cancer patients with respiratory symptoms such as fever, cough, or difficulty breathing between February 1st and February 28th, 2023. Only specimens with significant bacterial growth were included; namely those samples that yielded moderate to heavy growth of the bacteria where it was the only bacterial pathogen in patients who had symptoms of respiratory infection. The study was approved by the institutional ethical committee (KMIO/MEC/2024/12/F/MO-64). Since the study was qualitative in nature, no statistical analysis was performed.

Identification of *S. maltophilia* cluster. We observed a significant and unexpected increase in *S. maltophilia* respiratory infections in our microbiology laboratory during February 2023. Enhanced surveillance and expedited biochemical testing revealed a cluster of multiple *S. maltophilia* cases in a row during February, with the last case occurring in early March 2023. Following this cluster, no additional *S. maltophilia* respiratory infections were identified. We conducted a retrospective review of patients with *S. maltophilia* respiratory infections during this period, collecting demographic, clinical, and microbiological data from their medical files for our study.

Microbiological identification and characterization. All bacterial isolates underwent a panel of five biochemical tests for initial genus-level identification, consisting of triple sugar iron agar, mannitol motility medium, indole, citrate utilization, and oxidase tests [7]. Following these tests, we assessed the motility of presumptive *S. maltophilia* isolates using hanging drop preparation. The isolates were then confirmed as *S. maltophilia* using the Vitek® 2 automated identification system (bioMérieux) according to the manufacturer's instructions.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing (AST) was performed on all *S. maltophilia* isolates using the Kirby-Bauer disk diffusion method. The following antimicrobial agents were tested: ceftazidime 30 µg, cefepime 30 µg, ciprofloxacin 5 µg, cefoperazone-sulbactam 75/30 µg, tigecycline 15 µg, colistin 10 µg, carbapenems (imipenem 10 µg, meropenem 10 µg, ertapenem 10 µg), aminoglycosides (gentamicin 10 µg, amikacin 30 µg, tobramycin 10 µg), piperacillin-tazobactam 100/10 µg, (Microxpress, Tulip Diagnostics, India) and trimethoprim-sulfamethoxazole 1.25/23.75 µg (HiMedia, Mumbai, India), according to the Clinical and Laboratory Standards Institute (CLSI) M100-S33 (2023) guidelines, using zone diameter breakpoints for non-fermenting Gram-negative bacteria [8].

Additional phenotypic characterization. A selection of *S. maltophilia* isolates underwent further phenotypic characterization, in addition to routine identification and antimicrobial susceptibility testing. These additional tests included DNase activity, lysine decarboxylase, gelatin liquefaction, and esculin hydrolysis assays [1, 7]. These supplementary tests were conducted on selected *S. maltophilia* isolates to provide a more comprehensive phenotypic profile, as they are not routinely performed for Gram-negative bacilli in our laboratory.

Biofilm formation assay. A selection of *S. maltophilia* isolates were assessed for biofilm formation using the standardized tube method [9]. Briefly, isolates were inoculated into brain-heart infusion (BHI) broth supplemented with 2% sucrose and incubated at 37°C for 24 h. Following incubation, the tubes were decanted, rinsed with phosphate-buffered saline (PBS) and air-dried. Adherent biofilms were stained with crystal violet. A biofilm was considered positive if a visible film lined the tube walls and bottom. An in-house *P. aeruginosa* isolate that had been previously characterized for biofilm production using the above tube method served as a positive control, while an uninoculated BHI broth served as a negative control.

RESULTS

Bacterial isolates and identification. Between February 1st and February 28th, 2023, our microbiology laboratory observed a significant increase in the isolation of oxidase-negative, non-glucose-fermenting, Gram-negative bacilli with greenish pigmentation from respiratory specimens. This increase was notable, with a total of 10 isolates identified during this period. Subsequent biochemical testing and automated identification methods confirmed all 10 isolates as *S. maltophilia* (Table 1).

Patient characteristics. The ten patients with *S. maltophilia* comprised six females and four males, with a median age of 42 years (range: 15-61). Eight patients had hematological cancers (six with acute leukemia and two with lymphoma), while two had solid tumors. These patients were admitted to various wards within the hospital, indicating sporadic cases.

Microbiological identification and characterization. Initial identification of all ten isolates was based on colony morphology and standard biochemical tests. The characteristic greenish pigmentation observed on chocolate and blood agar (Figure 1), combined with biochemical tests, suggested *S. maltophilia*, which can sometimes resemble certain *Pseudomonas* species.

[DOI: 10.61186/JoMMID.13.1.50]

Biochemical testing revealed that the isolates were non-glucose-fermenters and oxidase-negative, which distinguished them from *Pseudomonas* species, which are typically oxidase-positive. The isolates were negative for indole production but positive for citrate utilization. On semi-solid mannitol motility medium, the isolates did not ferment mannitol, but motility was confirmed by hanging drop preparation. Although an oxidase-negative result could lead to misidentification as *Acinetobacter* species, subsequent hanging drop preparations confirmed motility in all isolates. Moreover, Gram staining revealed Gram-negative bacilli, distinguishing them from the typical Gram-negative coccobacilli morphology of *Acinetobacter* species. All ten isolates were presumptively identified as *S. maltophilia* based on these initial biochemical characteristics and further confirmed using the Vitek® 2 system (bioMérieux).

Additional phenotypic characterization. A selection of seven subcultured isolates exhibited DNase activity, lysine decarboxylation, and gelatin liquefaction. Six of these isolates also demonstrated esculin hydrolysis. These additional phenotypic characteristics further supported the identification of the isolates as *S. maltophilia*. These phenotypic tests may be useful alternatives for identifying *S. maltophilia* when automated identification systems are unavailable. Moreover, biofilm formation was observed in all seven isolates tested, providing further insight into their phenotypic characteristics.

Antimicrobial susceptibility testing. The antimicrobial susceptibility profiles of the *S. maltophilia* isolates are depicted in Figure 2. As *S. maltophilia* is naturally resistant to carbapenems, aminoglycosides, and piperacillin-tazobactam, these agents were tested, but their results are not shown [8]. Furthermore, all isolates exhibited susceptibility to trimethoprim-sulfamethoxazole, and 90% were susceptible to ciprofloxacin.

Table 1. Clinical and demographic characteristics of ten patients with respiratory cultures positive for *S. maltophilia*

No.	Age/Sex	Diagnosis	Sample	Sampling date (day/month/year)	Other microbes isolated	Ward
1	15/Female	Hodgkin's lymphoma	Sputum	5.2.23	-	Female Medical ward
2	40/Female	Mantle cell lymphoma	Sputum	5.2.23	-	Outpatient Department
3	23/Male	Acute lymphoblastic leukemia	Throat swab	6.2.23	*Candida* (moderate)	Male Medical ward
4	30/Female	Acute lymphoblastic leukemia	Throat swab	14.2.23	*Staphylococcus aureus* (Heavy)	Medical ICU
5	26/Male	Acute lymphoblastic leukemia	Throat swab	14.2.23	-	Outpatient Department
6	60/Female	Oral carcinoma (solid tumor)	Throat swab	14.2.23	*Klebsiella pneumoniae* (Heavy)	Post op ICU
7	61/Male	Acute myeloid leukemia	Throat swab	15.2.23	-	Medical ICU
8	60/Male	Carcinoma lung (solid tumor)	Broncho-alveolar Lavage	16.2.23	-	Surgical ward
9	17/Female	Acute myeloid leukemia	Sputum	21.2.23	-	Medical ICU
10	24/Female	Acute myeloid leukemia	Sputum	2.3.23	-	Medical ICU

[DOI: 10.61186/JoMMID.13.1.50]

Fig. 1. Characteristic lavender-green colonies of *S. maltophilia* on blood agar

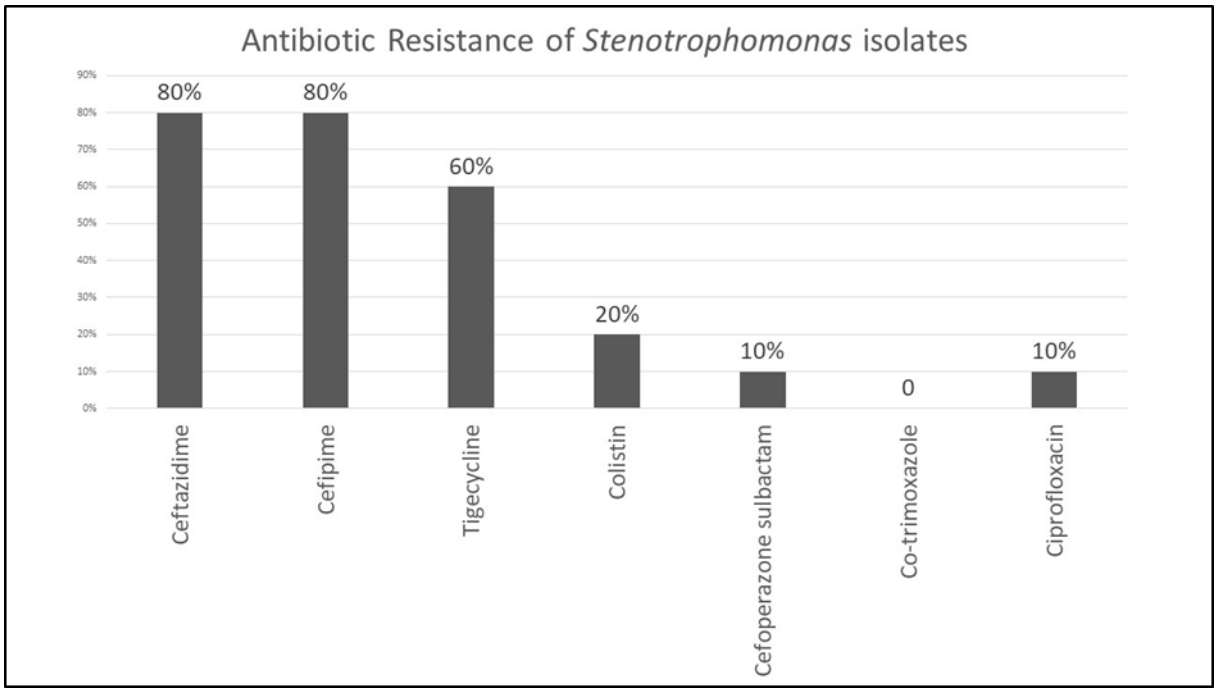

Fig. 2. Antimicrobial susceptibility profiles of ten *S. maltophilia* isolates.

[DOI: 10.61186/JoMMID.13.1.50]

DISCUSSION

Recent decades have witnessed a rise in *S. maltophilia* infections, particularly among immunocompromised patients, such as those with cancer [10-14]. This trend is likely multifactorial, driven by factors such as the growing number of patients with compromised immune systems due to advances in cancer treatment and transplantation medicine, increased antimicrobial use, and the widespread utilization of invasive medical devices [10,15]. Furthermore, several reports from India and elsewhere have highlighted the emergence of *S. maltophilia* as a significant pathogen within the country [4, 16-19], emphasizing its growing clinical relevance. In this study, we found 10 cases of *Stenotrophomonas* causing respiratory infection within a short span of a month.

Established risk factors for *S. maltophilia* infections include underlying malignancy, prolonged hospital stays, intensive care unit (ICU) admission, neutropenia, presence of indwelling devices (*e.g.*, central venous catheters), and exposure to broad-spectrum beta-lactam antimicrobials, particularly carbapenems, to which the organism is intrinsically resistant [1, 10, 14, 15, 20]. In our study, eight patients presented with hematological malignancies (six with acute leukemia and two with lymphoma), while two had solid tumors. Increased incidence of *Stenotrophomonas* infections in ICUs is known [21]. In our study, 50% of the patients were from ICU, and the majority had undergone multiple cycles of anticancer chemotherapy and experienced frequent hospitalizations. Interestingly, none of the patients in this study were mechanically ventilated, and most had only peripheral venous catheters as indwelling devices.

S. maltophilia respiratory infections, including pneumonia, have been associated with mortality rates ranging from 21% to 77% [4, 11, 21]. In the context of cancer patients, *S. maltophilia* respiratory infections have been reported to carry a mortality rate of up to 50% in untreated or severe cases [12, 14]. In our study, however, all ten patients responded favorably to appropriate and timely antimicrobial therapy and recovered from their *S. maltophilia* respiratory infections, highlighting the importance of prompt and effective treatment in this patient population.

Within healthcare settings, *S. maltophilia* demonstrates the ability to adhere to and form biofilms on a range of medical devices, including urinary catheters, ventilators, endoscopes, nebulizers, and central venous catheters, contributing to nosocomial infections [4, 5, 16, 22, 23]. Consistent with this, all seven tested *S. maltophilia* isolates in our study exhibited biofilm formation, a key virulence factor contributing to enhanced antimicrobial resistance and challenging treatment outcomes. However, despite the biofilm-forming capability of the isolates, all patients in this cohort responded favorably to prompt and appropriate antimicrobial therapy.

Trimethoprim-sulfamethoxazole (TMP-SMX) remains the mainstay of treatment for S. *maltophilia* infections, either as monotherapy or in combination with a fluoroquinolone such as levofloxacin [6, 11]. Levofloxacin may also be considered as monotherapy, particularly for infections such as pneumonia. Alternative therapeutic options include ticarcillin-clavulanate, aerosolized colistin, tetracyclines, tigecycline, and the newer cephalosporin, cefiderocol [1, 6, 11]. Notably, although resistance to TMP-SMX has been reported in *S. maltophilia* [6], all isolates in our study were susceptible to TMP-SMX, and the affected patients responded favorably to treatment with this antibiotic.

Distinguishing *S. maltophilia* infection from colonization or contamination can be challenging [5, 6]. In this study, all ten patients presented with respiratory symptoms such as fever, cough, and/or dyspnea. *S. maltophilia* was isolated in heavy growth from nine patients and in moderate growth as the sole potential pathogen from one patient with acute leukemia. In seven patients (70%), *S. maltophilia* was the sole potential pathogen, while in three patients, cultures also yielded other pathogens, including *Candida* spp., *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Given the immunocompromised status of all patients, antimicrobial therapy targeting *S. maltophilia* was deemed appropriate, even in cases with co-infection. Consequently, all patients responded favorably to TMP-SMX therapy directed against *S. maltophilia*. These findings support the pathogenic role of *S. maltophilia* in causing infections among cancer patients.

The SENTRY Antimicrobial Surveillance Program (1997-2008) reported a 3% incidence of *S. maltophilia* in nosocomial pneumonia cases [24]. Similarly, a 2009 study from South India found *S. maltophilia* accounted for 2.5% of non-fermenting Gram-negative bacilli isolated from a range of clinical specimens, including respiratory samples [25]. The present series of ten cases likely represented a cluster of sporadic *S. maltophilia* infections rather than a true outbreak, as there was no evidence of a common source or patient-to-patient transmission [26].

S. maltophilia infections typically manifest as nosocomial infections acquired directly from contaminated water systems, inadequately sterilized equipment, or other healthcare-related sources, with person-to-person transmission being uncommon [5, 6, 20]. Moreover, the use of broad-spectrum antibiotics, especially carbapenems, can exert selective pressure, leading to the overgrowth of *S. maltophilia* from the patient's pre-existing commensal flora [5]. In our study, the *S. maltophilia* infections may have originated from contact with contaminated devices or equipment. Furthermore, as most patients received empirical broad-spectrum antibiotics, such as carbapenems and cephalosporins, selective pressure could have contributed to the emergence of *S. maltophilia* infections. The distribution of infections across multiple wards without

[DOI: 10.61186/JoMMID.13.1.50]

consistent temporal clustering argues against a common source, and there was no evidence of patient-to-patient transmission.

In response to the observed cluster of *S. maltophilia* infections, comprehensive infection control measures were intensified across all wards under the active supervision of our infection control team. These measures included regular ward inspections with emphasis on adherence to standard and contact precautions, hand hygiene practices, proper disinfection of medical devices and equipment, frequent replacement of disinfectant and irrigation solutions, aseptic handling and storage of sterile supplies, water safety and disinfection protocols, general housekeeping and cleanliness, appropriate biomedical waste management, and other relevant infection control practices. Special focus was given to high-touch surfaces and patient-contact areas, including masks, suction apparatus, humidifiers, and cannulas, which are prone to moisture accumulation and potential contamination. Before active surveillance sampling of potential environmental sources (*e.g.*, solutions, equipment surfaces, water sources) could be initiated to identify the likely origin of the cluster, the *S. maltophilia* infections ceased. Such surveillance could have provided valuable insights into the source and informed future preventive strategies to mitigate similar clusters or outbreaks.

A 2020 study from Sweden investigating a cluster of four *S. maltophilia* respiratory infections in ICU patients utilized environmental sampling and whole-genome sequencing to identify sinks as the source of infection [20]. Timely active surveillance and source identification in that study successfully prevented further *S. maltophilia* infections. A recent study from Italy on an outbreak in seven patients within the same ICU used environmental sampling and multilocus sequence typing or MLST to conclude that the transmission may have occurred by cross contamination during patient care [21]. A limitation of our study is its observational design, which precluded the implementation of timely active surveillance measures. Future studies should incorporate prospective environmental sampling, molecular typing (*e.g*., whole-genome sequencing or MLST), and real-time surveillance to enhance data collection, facilitate source identification, and inform effective infection control strategies. Nonetheless, reporting such clusters of unusual infections is crucial as it raises awareness among healthcare personnel regarding the importance of accurate identification and appropriate management of these pathogens within the constraints of existing infection control measures and infrastructure.

Reporting isolates solely as "other non-fermenting Gram-negative bacteria" is inadequate for guiding appropriate clinical management. Unlike many other non-fermenters, *S. maltophilia* exhibits intrinsic resistance to various antibiotics, including carbapenems. However, it remains susceptible to cost-effective options such as TMP-SMX. To optimize infection control practices, thorough and regular disinfection of surfaces, patient care devices, and equipment is essential. In our case, the abrupt onset and cessation of *S. maltophilia* infections without further cases in subsequent months suggest a cluster rather than a true outbreak, characterized by sporadic occurrence, absence of a point source, and lack of patient-to-patient transmission. Emerging molecular typing techniques such as amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), or whole-genome sequencing (WGS) can aid in identifying bacterial clones and tracing the source of infection. Moving forward, preparedness through prompt initiation of microbiological surveillance, coupled with rigorous adherence to infection control practices, is essential to effectively manage and prevent similar clusters or outbreaks.

ACKNOWLEDGEMENT

Mahua Sinha conceived and designed the study, identified the infections, conducted the literature search, performed the laboratory experiments, acquired and analyzed the data, and prepared the manuscript. Sahana Shetty, Priyanka Shivashankar, and Junia Joseph assisted with the literature search, laboratory experiments, data acquisition, and manuscript review. Sumathi Gurusiddappa contributed to the study design, literature search, and manuscript review. All authors contributed to and approved the final manuscript. No funding was received for this study.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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[DOI: 10.61186/JoMMID.13.1.50]

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Cite this article:
Sinha m, Gurusiddappa S, Shivashankar P, Joseph J, Shetty S. *Sporadic Stenotrophomonas* maltophilia Respiratory Infections in Cancer Patients. J Med Microbiol Infect Dis, 2025; 13 (1): 50-56. DOI: 10.61186/JoMMID.13.1.50.

[DOI: 10.61186/JoMMID.13.1.50]